PB83-163436

Determination of Background Levels of Lead and Cadmium in Raw Agricultural Crops by Using Differential Pulse Anodic Stripping Voltammetry

(U.S.) Food and Drug Administration
Cincinnati, OH

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1982

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO.	2.	3.
EPA-600/J-82-277	JOURNAL ARTICLE	PB83-163436

4. TITLE AND SUBTITLE	5. REPORT DATE
JOURNAL ARTICLE: Determination of Background Levels of Lead and Cadmium in Raw Agricultural Crops by Using Differential Pulse Anodic Stripping Voltammetry	1982
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R.D. Satzger, C.S. Clow, E. Bonnin, F.L. Fricke	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Food and Drug Administration Elemental Analysis Research Center Cincinnati, Ohio 45202	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. IAG AD-12-F-1-513-0
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory U.S. Environmental Protection Agency Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Reference: J. Assoc. Off. Anal. Chem., 65(4):987-991, 1982

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	6
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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EPA-600/J-82-277
JOURNAL ARTICLE

JOURNAL ARTICLE:
pp. 987-991

Determination of Background Levels of Lead and Cadmium in Raw Agricultural Crops by Using Differential Pulse Anodic Stripping Voltammetry

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A method is described for the simultaneous determination of ultratrace levels of lead and cadmium in selected agricultural crop samples by differential pulse anodic stripping voltammetry. Samples are dry ashed at high temperature with H_2SO_4 as an ashing aid. Techniques are described to control the lead and cadmium blank levels of 2 ng and 0.4 ng, respectively. Typical relative standard deviations for the crop analyses are 13% at 100 ng/g and 25% at 10 ng/g for lead, and 5% at 100 ng/g and 10% at 10 ng/g for cadmium. The lowest quantifiable level, based on 3 g dry sample, is 2 ng/g for lead and 1 ng/g for cadmium. Recovery studies, precision studies, and analyses of NBS Standard Reference Materials demonstrate the accuracy and reproducibility of this technique. A summary of results for over 1700 crop samples is reported.

The symptoms and pathology of lead and cadmium poisoning are well documented (1, 2). However, concern has recently arisen over the health effects of long term low level exposure to these elements. Much of the lead and cadmium data at these levels fail to distinguish between actual levels of analyte in a sample and levels which have been elevated by methods of sample collection, handling, and analysis. This elevation had led to values in foods which are so variable as to be meaningless (3). This has prompted the investigation of methodology which leads to accurate, precise results at ultratrace levels.

Conventional methods have involved a 2-step process comprised of the destruction of the organic matrix followed by analyte determination. In the first step, a dry ash method is generally preferred which lowers the possibility of sample contamination by using small volumes of reagents. Volatilization losses are circumvented by proper choice of ash aid and ashing temperatures (4). In the determinative step, both differential pulse anodic stripping voltammetry (DPASV) and graphite furnace atomic absorption spectrometry (GFAAS) are used routinely (5–9). The detection limits for both techniques are governed by the levels of analyte in the reagent blank.

The methodology presented here is a modification of previous work (10) which has proven to be unreliable. This method combines the use of less sulfuric acid as an ashing aid, elevated ashing temperatures, and shorter ash times to provide an ashing method which optimizes recoveries and minimizes reagent blanks. Stringent contamination controls for Pb and Cd have also been instituted, resulting in increased precision and accuracy. DPASV provides a method of simultaneous low level determination. This paper presents the controls and methodology used to achieve accurate, meaningful, ultratrace analyses of lead and cadmium.

Presented at the Symposium on Analytical Methodology for Lead in Foods, 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, at Washington, DC.

METHOD

Principle

Freeze-dried agricultural crops are dry ashed at 500°C using 40% H_2SO_4 as an ash aid and diluted to volume with 4% HNO_3. An aliquot is analyzed for Pb and Cd by DPASV.

Apparatus

(a) *Polarograph.*—Princeton Applied Research (PAR) Model 384, or equivalent, equipped with Model 303 static mercury dropping electrode and Model 305 stirrer (Princeton, NJ 08540).

(b) *X-Y recorder.*—Hi-Plot digital plotter (Houston Instruments, Austin, TX 78753, or equivalent).

(c) *Electrolyte purification apparatus.*—PAR Model 9500, or equivalent.

(d) *Laboratory box furnace.*—Lindberg (Watertown, WI 53094) with microprocessor control (Model 73211, Research, Inc., Minneapolis, MN 55424, or equivalent).

(e) *Ashing vessels.*—Quartz tall-form beakers (Labcrest Scientific Glass, Los Angeles, CA 90022), 100 mm high X 50 mm id X 59 mm od with watch glass covers, or equivalent.

(f) *Freeze-dryer.*—18 L with shell freezer (Model 75018, Labconco Corp., Kansas City, MO 64132, or equivalent).

Table 1. Standard recoveries[a] and ashing parameters

Sample	Dry wt, g	Vol. H_2SO_4, mL	Hours at 500°C	Spike level, μg/g	Mean recovery, % Cd	Mean recovery, % Pb
Lettuce	1.0	4	12	0.4	98 ± 13	96 ± 12
Peanuts	2.0	3	20	0.05	99 ± 12	100 ± 19
Potatoes	2.0	3	8	0.1	98 ± 12	101 ± 12
Soybeans	2.0	3	12	0.1	94 ± 11	95 ± 10
Sweet corn	3.0	4	8	0.03	95 ± 14	98 ± 18
Wheat	3.0	3	20	0.07	99 ± 14	103 ± 16

[a] Based on 20 determinations.

(g) *Food cutter.*—Model 8181 (Hobart Corp., Troy, OH 45374), coated with Kynar (vinylidene fluoride resin, Pennwalt Corp., Philadelphia, PA), or equivalent.

(h) *Clean air equipment.*—Environmental Air Control, Inc. (Hagerstown, MD 21740), or equivalent.

(i) *Blender*—Sears, Roebuck and Co. (Chicago, IL 60684), or equivalent.

Reagents

(a) *Distilled deionized water.*—18 Megohm cm^{-1} (DDW) (Millipore Corp., Bedford, MA 01730).

(b) *Sulfuric acid, nitric acid.*—J.T. Baker Chemical Co. (Phillipsburg, NJ 08865), "Ultrex" for dry ash; "Instra Analyzed" HNO_3 for glassware preconditioning.

(c) *Buffer/electrolyte.*—pH ca 4.6. Dissolve 102 g anhydrous sodium acetate, 8 g tartaric acid, and 97 mL glacial acetic acid (ACS reagent grade) and dilute to 1.0 L with DDW.

(d) *Standard solutions.*—Prepared daily from 1000 μg/mL stock standards of Pb and Cd (Spex Industries, Inc., Metuchen, NJ).

(e) *Nitrogen.*—High purity, 99.998% (Liquid Carbonic, Cincinnati, OH). Passed through oxygen scrubbing solution prepared by boiling 2 g ammonium metavanadate in 25 mL HCl (7).

(f) *Mercury.*—Triple-distilled (Bethlehem Apparatus Corp., Hellertown, PA).

Sample Collection

Site selection and sampling were critical parts of the Pb and Cd study. Agricultural sites selected represent average conditions for crop and soil series. The following criteria were used in site selection: The site was more than 5 miles downwind from a coal-fired power plant or similar large fossil fuel plant, at least 200 m from a highway and 100 m from a lightly traveled road, 100 m from a current, abandoned, or known obliterated building site, and 50 m from end rows or other areas where large quantities of fertilizer could have been stockpiled. Equipment used in sampling was plasticware and samples were transported in sealed plastic containers.

Preparation of Sample Composite

Perform all sample preparation steps in a clean-air environment. Wear disposable polyethylene gloves during all sample handling. Reduce samples to their edible portion, wash with DDW, and air dry. Homogenize in a food cutter. Place samples with high water content (sweet corn, lettuce, potatoes) in acid-washed borosilicate glass freeze-dry flasks, shell-freeze at −50°C, and freeze-dry. Pulverize dried samples in a blender to ca 40 mesh and store in acid-washed linear polyethylene bottles. (*Note:* This is the only stage in which metallic equipment contacts the samples.) Soak wheat and peanut samples in DDW overnight before freeze-drying to facilitate grinding.

Final sample composites represented 5 heads lettuce, 10 ears sweet corn, 80 pods soybeans, 10 potatoes, 200 heads wheat, and 200 peanuts.

Glassware Preconditioning

Immediately before digestion, condition all glassware as follows: Rinse 5 times with DDW. Soak in hot (60°C) 30% HNO_3 for 2 h. Rinse 5 times with DDW. Rinse with concentrated HNO_3. Rinse 5 times with DDW. Store glassware dedicated to Pb and Cd analyses in a clean-air environment.

Sample Digestion

Weigh 1–3 g freeze-dried sample into ashing vessel and spread as thinly as possible to allow maximum access of oxygen. Add 3–4 mL of 40% (v/v) H_2SO_4, depending on sample matrix, and an equal volume of DDW. Carefully agitate all samples to ensure thorough wetting. Cover vessels with watch glasses and place in furnace

Table 2. Instrumental parameters for DPASV determination of lead and cadmium

Initial potential	0.800 V vs Ag/AgCl
Final potential	−0.320 V
Purge time[a]	300 s
Scan rate	4 mV/s
Deposition time	120 s
Pulse modulation	50 mV
Current sensitivity[b]	20 nA full scale
Drop size	medium
Stirring rate	400 rpm

[a] Purge with oxygen-free nitrogen saturated with electrolyte (11).
[b] Sensitivity commensurate with concentration.

at 115°C for 3 h to evaporate any water. Ramp temperature over 1 h period to 275°C and hold for 3 h; ramp over 2 h period to 500°C and hold for 8–20 h, depending on sample type (Table 1). Remove ashed sample from oven and cool to room temperature in clean-air hood. Add 1 mL HNO_3 and 0.5 mL DDW to ashing vessels; evaporate to dryness on hot plate (250°C) in clean-fume hood. Exercise caution to prevent bumping. Place sample in furnace at 200°C and elevate to 450°C for ca 30 min. Digestion is complete when a carbon-free white ash is obtained. Repeat HNO_3 treatment, if necessary. Store ashed samples in clean-air environment before determination.

Electrochemical Determination

Solubilize sample residue in 1 mL HNO_3 and 5 mL DDW. Warm ashing vessel on hot plate (120°C) to remove adsorbed analyte. Quantitatively transfer contents of ashing vessel to 25 mL volumetric flask, dilute to volume with DDW, and shake thoroughly. Let insoluble material, if present, settle before voltammetric determination.

Transfer 5.0 mL sample solution to polypropylene cell containing 5.0 mL pre-electrolyzed HOAc/NaOAc buffer-electrolyte. Transfer acid-washed Teflon stirring bar (12.7 × 3 mm) to cell. Clamp cell in place over stirring motor (solution pH, ca 4.0). Stirring is controlled by microprocessor. Instrumental parameters are listed in Table 2. Purge-stir for 30 s between successive runs after initial 5 min purge.

Determination

Quantitate analyte levels by method of additions of μL volumes of mixed standard solution to sample cell. Determine peak amplitudes (nA) with respect to a tangent fitted to the base of the peak. Calculate analyte concentration from resulting peak amplitudes and corresponding amount of standard added (μg), using a linear regression analysis. The analyte level is the absolute value of the X-axis intercept.

Results and Discussion

Initial experiments confirmed that the levels of Pb and Cd in the laboratory environment were too high to perform a background study, and upgrading was required (12). Contamination originated from the laboratory atmosphere, slate bench tops, conventional fume hoods, and glassware, including volumetric ware and polarographic cells. Transfer of purified electrolyte to storage containers also added to the total background signal. The level of Pb in the laboratory resulted in sporadic contamination at the ppm level.

Clean-air modules were installed, bench tops were cleared of metal fixtures and covered with polyvinylchloride sheets, and a small clean-fume hood was constructed to eliminate airborne particulates falling from the conventional fume hood. The clean-fume hood consisted of modifying a conventional fume hood to accept a small laminar flow clean hood. Purified buffer was

Table 3. Determination of lead and cadmium in NBS Standard Reference Materials by DPASV

	Mean,[a] μg/g		NBS, μg/g	
Sample	Cd	Pb	Cd	Pb
Wheat	0.029 ± 0.004	0.018 ± 0.003	0.032 ± 0.007	0.020 ± 0.010[b]
Rice	0.025 ± 0.002	0.035 ± 0.004	0.029 ± 0.004	0.041 ± 0.010[b]
Spinach	1.23 ± 0.16	1.1 ± 0.08	(1.5)[c]	1.2 ± 0.20
Orchard Leaves	0.100 ± 0.004	44.9 ± 1.0	0.110 ± 0.020	45.0 ± 3.0
Tomato Leaves	2.74 ± 0.20	5.6 ± 0.20	(3)[c]	6.3 ± 0.3
Oyster Tissue	3.24 ± 0.29	0.51 ± 0.06	3.5 ± 0.40	0.48 ± 0.04

[a] Four separate determinations.
[b] Preliminary NBS data.
[c] Non certified NBS values.

Table 4. Accuracy of lead and cadmium DPASV determinations at low levels (NBS Wheat) [a]

	Weight, g	Cd, ng/g	Pb, ng/g
Sample A	0.152	25.2	20.2
Sample B	0.113	28.7	19.6
Sample C [b]	2.00	34.0 ± 4.0 [c]	15.0 ± 2.0 [c]

[a] NBS values: Cd 32 ± 7 ng/g, Pb 20 ± 10 ng/g.
[b] Contamination check: through entire procedure including compositing, freeze-drying, dry ash.
[c] Mean value from 3 separate determinations.

Table 6. Precision of Cd duplicates

Sample Cd content, ng/g	No. of duplicates [a]	Range of dev., % [b]	Mean dev., %
<10	12	2–37	13.7
10–50	40	1–36	9.4
51–100	45	1–27	9.6
101–500	49	1–21	6.0
>500	9	1–16	4.5

[a] Number of samples determined in separate duplicate analyses.
[b] % deviation = [(difference between Cd content of duplicate analyses)/(mean Cd content)] × 100.

Table 7. Precision of Pb duplicates

Sample Pb content, ng/g	No. of duplicates [a]	Range of dev., % [b]	Mean dev., %
<10	20	3–90	34.2
10–50	60	1–80	20.4
51–100	34	1–48	15.1
101–500	27	1–32	11.9
>500	9	1–16	6.9

[a] Number of samples determined in separate duplicate analyses.
[b] % deviation = [(difference between Pb content of duplicate analyses)/(mean Pb content)] × 100.

stored in the pre-electrolysis apparatus along with a pipet used in transfer. Acids used in the dry ash method were evaluated for their contribution to the analyte signal. The ultrapure acids used lowered the Pb blank values from 10 to 2 ng.

Mean values for the reagent blank based on 30 determinations are 1.8 ± 0.6 ng for Pb and 0.4 ± 0.3 ng for Cd. The detection limit calculated as twice the standard deviation of the reagent blank is 1.1 ng for Pb and 0.6 ng for Cd. The lowest quantifiable level (LQL), defined as 5 times the detection limit, is 6 ng for Pb and 3 ng for Cd. The sample detection limits can be decreased by using larger amounts of freeze-dried samples and by reducing the dilution factor. Up to 10 g dry sample has been digested by this method.

The accuracy of the method was certified by standard recoveries, analysis of NBS Standard Reference Materials, and participation in a WHO-FAO quality assurance program. Data from 25 reagent blanks fortified with 0.1 ng of mixed Pb and Cd standard resulted in quantitative recovery of both elements: Pb, 99 ± 14% and Cd, 97 ± 10%. Recoveries of standard spikes added to crop samples are listed in Table 1. The spike levels were commensurate with the analyte levels in the samples. This also shows the ashing time and volume of ashing aid which were optimized for each crop type. The results obtained in determinations using NBS materials are given in Table 3. With the exception of tomato leaves, all of the values obtained for these materials fall within the range of the NBS certified values. The value for Pb in tomato leaves is low. NBS noted that this could be a homogeneity problem. This method was verified in a WHO-FAO analytical quality assurance program for monitoring Pb and Cd in which 37 laboratories in 13 countries participated. NBS SRMs were used in this study. Each laboratory obtained results by its own method, and these results were evaluated on the basis of their proximity to an expected value. Using the method described, our Cd results were nearest to the expected values, and Pb

Table 5. Precision of sample analyses

Sample	No. of replicate detns	Mean, ng/g		% RSD	
		Cd	Pb	Cd	Pb
Corn	8	7.3 ± 0.9	59 ± 8.0	11.9	13
Lettuce	9	540 ± 28	160 ± 14	5.2	8.6
Peanuts	8	110 ± 7.0	6.4 ± 2.0	6.6	31
Potatoes	10	66 ± 3.9	6.0 ± 2.0	5.9	32
Soybeans	9	120 ± 15	110 ± 15	4.0	14
Wheat	8	13 ± 1.0	13 ± 3.0	7.6	23

Table 8. National levels of Cd and Pb in raw agricultural crops[a]

Crop type	No. of samples	Range, ng/g		Median, ng/g	
		Cd	Pb	Cd	Pb
Corn	242	<1–200	<2–250	10	10
Lettuce	166	30–3500	30–1500	350	200
Peanuts	322	10–700	<3–200	70	10
Potatoes	313	10–490	5–240	140	20
Soybeans	354	4–1000	4–1000	40	30
Wheat	322	5–200	<2–250	30	20

[a] Reported on a dry weight basis.

results were second nearest. This study verified accuracy at trace levels.

Ultratrace accuracy was verified (Table 4) by using a small amount of NBS Wheat which contained low levels of Pb and Cd. Based on a 0.1 g sample, solution concentrations of 0.004 ng/mL for Pb and 0.006 ng/mL for Cd were obtained. Instrumental parameters were modified to increase sensitivity. Scan rate was slowed to 2 mV/s, deposition time was increased to 15 min, and the pulse height was increased to 100 mV. Using these parameters, the data obtained were in agreement with the reported NBS values. A contamination check at ultratrace levels for the entire method, including all sample preparation phases, confirmed that neither Pb nor Cd was gained or lost.

The precision of the method (Table 5) was estimated by multiple analyses of unspiked samples of 6 raw agricultural crop types investigated. A comparison of duplicates from all 6 crop types over a wide range of Cd and Pb concentrations (Tables 6 and 7, respectively) provides a general overview of the precision that can be expected in the routine application of this method.

The results for over 1700 raw agricultural crops are listed in Table 8. They are part of a continuing survey on background levels of Pb and Cd in various crops collected from their growing sites throughout the United States.

Conclusion

The dry ash voltammetric method described here is a sensitive, accurate, precise procedure for determining trace and ultratrace levels of Pb and Cd in raw agricultural crops. Contamination control steps must be undertaken to obtain meaningful results at ultratrace levels.

References

(1) Underwood, E. J. (1977) *Trace Elements in Human and Animal Nutrition*, 4th Ed., Academic Press, New York, NY, Chapters 9, 17
(2) Ochiai, E. (1977) *Bioinorganic Chemistry, An Introduction*, Allyn and Bacon, Inc., Boston, MA, Sections 17.2.2, 17.2.3
(3) Settle, D. M., & Patterson, C. C. (1980) *Science* **207,** 1167–1176
(4) Gorsuch, T. T. (1970) *The Destruction of Organic Matter*, Pergamon Press, New York, NY, pp. 93–99
(5) Holak, W. (1975) *J. Assoc. Off. Anal. Chem.* **58,** 777–780
(6) Chittleborough, G., & Steel, B. J. (1980) *Anal. Chim. Acta* **119,** 235–241
(7) Poldoski, J. E. (1980) *Anal. Chem.* **52,** 1147–1151
(8) Dabeka, R. W. (1979) *Anal. Chem.* **51,** 902–907
(9) Fiorino, J. A., et al. (1973) *J. Assoc. Off. Anal. Chem.* **56,** 1246–1251
(10) Jones, J. W., Gajan, R. J., Boyer, K. W., & Fiorino, J. A. (1977) *J. Assoc. Off. Anal. Chem.* **60,** 826–832
(11) Princeton Applied Research Corp. (1974) Application Note AN108A-7.5M-12/74-MG, Princeton, NJ
(12) Zief, M., & Mitchell, J. W. (1976) *Contamination Control in Trace Element Analysis*, John Wiley and Sons, New York, NY